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Association Between Chromosome 9p21 Variants and the Ankle-Brachial Index Identified by a Meta-Analysis of 21 Genome-Wide Association Studies

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Background—Genetic determinants of peripheral arterial disease (PAD) remain largely unknown. To identify genetic variants associated with the ankle-brachial index (ABI), a noninvasive measure of PAD, we conducted a meta-analysis of genome-wide association study data from 21 population-based cohorts.

Methods and Results—Continuous ABI and PAD (ABI ≤0.9) phenotypes adjusted for age and sex were examined. Each study conducted genotyping and imputed data to the ≈2.5 million single nucleotide polymorphisms (SNPs) in HapMap. Linear and logistic regression models were used to test each SNP for association with ABI and PAD using additive genetic models. Study-specific data were combined using fixed effects inverse variance weighted meta-analyses. There were a total of 41 692 participants of European ancestry (≈60% women, mean ABI 1.02 to 1.19), including 3409 participants with PAD and with genome-wide association study data available. In the discovery meta-analysis, rs10757269 on chromosome 9 near *CDKN2B* had the strongest association with ABI ($\beta = -0.006$, $P = 2.46 \times 10^{-8}$). We sought replication of the 6 strongest SNP associations in 5 population-based studies and 3 clinical samples (n=16 717). The association for rs10757269 strengthened in the combined discovery and replication analysis ($P = 2.65 \times 10^{-9}$). No other SNP associations for ABI or PAD achieved genome-wide significance. However, 2 previously reported candidate genes for PAD and 1 SNP associated with coronary artery disease were associated with ABI: *DAB21P* (rs13290547, $P = 3.6 \times 10^{-5}$), *CYBA* (rs3794624, $P = 6.3 \times 10^{-5}$), and rs1122608 (*LDLR*, $P = 0.0026$).

Conclusions—Genome-wide association studies in more than 40 000 individuals identified 1 genome wide significant association on chromosome 9p21 with ABI. Two candidate genes for PAD and 1 SNP for coronary artery disease are associated with ABI. **(*Circ Cardiovasc Genet*. 2012;5:100-112.)**

Key Words: cohort study ■ genetic association ■ genome-wide association study ■ meta-analysis ■ peripheral vascular disease

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Peripheral arterial disease (PAD) affects approximately 27 million people in Europe and North America,[1] and is associated with increased risk for myocardial infarction, stroke, and mortality.[2–6] Measurement of ankle and arm blood pressures with a Doppler device and calculation of the ankle-brachial index (ABI) is a simple and reliable method to detect PAD. An ABI ≤0.90 is indicative of definite PAD.[7] In previous work, the Ankle-Brachial Index Collaboration demonstrated a reverse J-shaped relationship of ABI with mortality and coronary events, with a low risk ABI ranging from 1.11 to 1.40.[8]

Clinical Perspective on p 112

Little is known about genetic susceptibility to PAD, but familial aggregation and heritability estimates suggest a significant genetic component.[9–13] A study of 112 biological candidate genes identified only 2 single nucleotide polymorphisms (SNPs) in *NOS3* significantly associated with ABI.[14] The candidate gene approach to identify novel genetic variants for PAD has been limited by modest study sample size, relatively small number of genes examined, and lack of replication in independent samples.[13]

Genome-wide association studies (GWAS) have led successfully to the discovery of novel genetic variants for several common diseases, including coronary artery disease (CAD).[15] The association between genetic variants on chromosome 9p21 and CAD has demonstrated replication,[16,17] persistent association across race or ethnicity,[18] and association with other vascular diseases.[19–21] Notably, GWAS of subclinical atherosclerosis phenotypes, such as intima-medial thickness or ABI, are sparse. Therefore, we conducted a meta-analysis of GWAS findings for ABI within an international consortium of 21 population-based cohort studies that included 41 692 participants of European ancestry, among whom 3409 participants had PAD (ABI ≤0.90). We conducted replication analyses of our strongest findings in over 16 000 individuals from population-based cohort studies and clinically based samples of PAD. We hypothesized that this approach would lead to the unbiased identification of genetic variants associated with ABI. Further, we hypothesized that some genetic variants for ABI would be identical to those reported to be associated with CAD or its risk factors given shared underlying biological pathways, while some genetic variants would be associated uniquely with PAD.

Methods

Discovery Studies

Our analyses were conducted within the international Cohorts for Heart and Aging Research in Genomic Epidemiology (CHARGE) Consortium,[22] and included 4 of the 5 original CHARGE cohorts: Atherosclerosis Risk in Communities Study (ARIC, n=7630), the Cardiovascular Health Study (CHS, n=3193), the Framingham Heart Study (FHS, n=3572), and the Rotterdam Study (RS-I, n=5169 and RS-II, n=1642). Ten additional population-based cohorts joined the collaboration for analysis of ABI phenotypes: the Family Heart Study (FamHS, n=1736), Genetic Epidemiology Network of Arteriopathy Study (GENOA, n=991), Gutenberg Heart Study (GHS, n=3122), Health, Aging, and Body Composition (Health ABC, n=1564), the Invecchiare in Chianti Study (InCHIANTI, n=1130), Cooperative Health Research in the Region of Augsburg (KORA F3, n=1581 and KORA F4, n=1407), Netherlands Study of Anxiety and Depression (NESDA, n=1612), Nijmegen Biomedical Study (NBS, n=544), and the Study of Health in Pomerania (SHIP, n=543). A further 6 studies derived from population isolates also were available for the analyses: Amish Study (Amish, n=1183), Croatia-Vis (n=897), Croatia-Korcula (n=851), Croatia-Split (n=499), Erasmus Rucphen Family Study (ERF, n=2133), and the Orkney Complex Disease Study (ORCADES, n=693). For all studies participating in the meta-analyses, each participant self-identified as European or European-American and provided written informed consent, and the Institutional Review Board at the parent institution for each respective cohort approved the study protocols. More detailed study-specific information is provided in the online-only Data Supplement Methods.

Ankle-Brachial Index Phenotypes

Ankle and brachial blood pressure measurements for each participating study were obtained from the baseline examination or the first examination in which the measurement was obtained. Details on the ABI protocol used and the calculation performed in each study are provided in online-only Data Supplement Table I. To calculate the ABI for each leg, the systolic blood pressure at each ankle was divided by the systolic blood pressure in the arm. If the systolic blood pressure was measured in both arms, the higher arm reading was used in the ABI calculation. If replicate readings were obtained, the mean of the 2 measurements for each limb was used to calculate the ABI, with the exception of InCHIANTI, which used the higher of the 2 readings of each measurement set to calculate the ABI. The lower of the ABIs from the 2 legs was used for analysis. In ARIC and FamHS, the ABI was measured in only 1 leg, chosen at random. Participants with an ABI >1.40 were excluded because this high ABI may represent medial sclerosis, fibrocalcific disease secondary to diabetes mellitus, or other causes of noncompressible vessels.

To maximize the sample size and the power to detect genetic variants with modest effects, and to examine the entire range of ABI values given the recent evidence of increased cardiovascular disease risk associated with ABI values up to 1.1,[8] we examined the continuous range of ABI <1.40. As a secondary analysis to provide a clinical phenotype, we defined PAD as ABI ≤0.90 and conducted a case (ABI ≤0.9)/control; ABI >0.90 and <1.40) comparison analysis.

Genotyping and Imputation

Different genotyping platforms were used by the 21 studies (online-only Data Supplement Table II). Each study imputed the genotype "dosage" (0 to 2) for the expected number of alleles for ≈2.5 million Phase II HapMap CEU SNPs for each participant using currently available imputation methods.[23] CHS used BIMBAM (available at http://stephenslab.uchicago.edu/software.html),[24] GHS, InCHIANTI, NESDA, and SHIP used IMPUTE,[25] and all other cohorts used MACH (http://www.sph.umich.edu/csg/abecasis/MaCH/).

Statistical Analysis

We devised a GWAS analysis plan for the ABI and PAD phenotypes that each study independently implemented. Sex-specific and age-adjusted residuals of ABI were created from linear regression models and used as phenotypes in the analysis. No transformation of the ABI measure was performed before analysis. In FHS, residuals also were obtained separately in the original and offspring cohorts. Multi-site studies (ARIC, CHS, and FamHS) additionally adjusted for field study site. Each SNP was tested for association with ABI in additive genetic models using linear regression. The Amish Study, FamHS, FHS, and GENOA cohorts used linear mixed effects models to account for familial correlations. Croatia-Vis, Croatia-Korcula, Croatia-Split, ERF, and ORCADES used the "mmscore" function of the GenABEL package for R statistical software for the association test under an additive model. This score test for a family-based association takes into account pedigree structure and allows unbiased estimations of SNP allelic effect when relatedness is present between examinees. Logistic regression adjusting for age and sex was used to test each SNP for association with the PAD phenotype. The FamHS,

FHS, and GENOA cohorts used generalized estimating equations clustering on family to account for family correlations.

A genome-wide meta-analysis using a fixed effects approach with inverse variance weighting was then conducted in METAL[26] [www.sph.umich.edu/csg/abecasis/metal] for 2 669 158 SNPs in the meta-analysis, excluding the population isolates (2 670 732 SNPs including the population isolates) that met imputation and quality control criteria (online-only Data Supplement Table II). Before meta-analysis, genomic control was applied to each study. The association of ABI per each additional risk allele was quantified by the regression slope (β), its standard error [SE(β)], and the corresponding probability value. We calculated a meta-analysis odds ratio for each of the most significant SNP associations for PAD. The meta-analysis odds ratio estimates the increase in odds of PAD for each additional copy of the risk allele of the SNP. SNP associations were considered to be significant on a genome-wide level at $P<5\times10^{8}$.[27,28] Standardized gene and SNP annotations were created using a PERL script.[29] We also tested for heterogeneity of study specific regression parameters using Cochran Q statistic. Because of concerns about heterogeneity, we conducted analyses of nonisolate studies and of the full group of studies. We selected SNPs for replication using results from the meta-analysis, excluding the population isolates, because the available replication samples did not include isolates. We excluded SNP association results if the total meta-analysis sample was less than 20 000 and if the average minor allele frequency of the SNP was <5%.

Replication

We sought to replicate independent SNP associations for ABI that attained genome-wide significance (1 region), SNPs with suggestive associations (5 regions, $P<10^{-5}$), and bioinformatics data supporting the signal. The bioinformatic analyses are described in detail in the online-only Data Supplement Material. In addition, we sought to replicate 1 SNP associated with both ABI and PAD at $P<10^{-4}$. The replication studies included 5 population-based studies and 3 clinically-based studies, including a total of over 16 000 participants: the Bruneck Study (n=786), the Copenhagen City Heart Study (CCHS, n=5330), the Multi-Ethnic Study of Atherosclerosis (MESA, n=2611), the National Health and Nutrition Examination Surveys (NHANES 1999–2002, n=2335), Prevention of Renal and Vascular End-stage disease (PREVEND, n=3691) cohort, Cardiovascular Disease in Intermittent Claudication (CAVASIC, n=443) Study, Genetic Determinants of Peripheral Arterial Disease (GenePAD, n=850), and the Linz Peripheral Arterial Disease (LIPAD, n=671) Study. Each collaborating study was provided with a SNP list and a detailed analysis plan. MESA and PREVEND used *in silico* genotyping (online-only Data Supplement Table II), and the remaining studies genotyped the SNPs using Taqman assays or Sequenom. Relative excess heterozygosity analysis demonstrated that all genotyped SNPs were compatible with Hardy-Weinberg equilibrium at the nominal 5% test-level (online-only Data Supplement Table III).[30]

Examination of Candidate Genes Associated With Peripheral Artery Disease and Coronary Artery Disease/Myocardial Infarction

We selected candidate genes for ABI or PAD from the published literature using PubMed search terms "([ankle-brachial index] OR [peripheral arterial disease]) AND polymorphism." Association studies with at least 100 cases and 100 controls were included regardless of whether the original study results were positive or negative. Using the discovery meta-analysis results for ABI, we then identified the most strongly associated SNPs based on probability values within the gene region ±100 kb upstream or downstream of the candidate gene. Because of the high correlation of imputed genotypes, the effective number of loci were calculated for each gene region[31] using the genotype scores from the KORA F4 Study (online-only Data Supplement Methods). Bonferroni correction of probability values then was applied in each region using the effective number of loci. Subsequently, false discovery rates (FDR) were calculated using these corrected probability values, accounting for the number of gene regions examined (online-only Data Supplement Methods). Lastly, we examined the association with ABI of 30 SNPs strongly associated with CAD in recent GWAS.[32–34] Our ABI discovery meta-analysis did not include 2 of the 30 SNPs (rs17465637 and rs3798220), and we were unable to identify proxy SNPs available in our data. Using the probability values for the 28 SNPs in our discovery meta-analysis, we then calculated the FDR for each CAD SNP, accounting for the 28 regions examined.

Results

Study Sample

The study sample included 41 692 participants of European ancestry (56% women, 6256 from population isolates) with ABI data and genome-wide genotyping. Participant characteristics at the time of ABI measurement for each cohort are provided in online-only Data Supplement Table IV. Across the studies the mean age ranged from 41.8 years to 73.8 years, the mean ABI ranged from 1.02 to 1.19, and 8.2% (n=3409) had PAD (ABI <0.9). Characteristics of the replication samples were similar to the discovery set (online-only Data Supplement Table V).

ABI-SNP Associations

We conducted a meta-analysis with (n=41 692) and without (n=35 434) the population isolates (online-only Data Supplement Figures I and II, QQ-plots and Manhattan plots, and study-specific lambdas ranged from 0.997 to 1.044). Our primary meta-analysis excluded studies from population isolates because of concern for study heterogeneity and the lack of availability of replication samples from isolates. The strongest SNP association for ABI was rs10757269 on chromosome 9 near *CDKN2B* ($\beta=-0.006$, $P=2.46\times10^{-8}$, P for heterogeneity=0.23, Table 1; meta-analysis results, including the population isolates, online-only Data Supplement Table VII). Among the 96 SNP associations for ABI with $P<10^{-5}$, 79 were located in the chromosome 9p21 region (online-only Data Supplement Table VI). The ABI SNP rs10757269 is in strong linkage disequilibrium (LD), with several SNPs in the region previously reported to be associated with CAD or myocardial infarction ($r^2>0.8$), but this ABI SNP is not in LD with SNPs previously associated with the type 2 diabetes mellitus (Figure 1). We repeated the meta-analysis to examine the association between ABI and rs10757269, first adjusting for CAD and then excluding individuals with CAD among the nonisolate studies. The association remained but was no longer genome-wide significant (adjusting for CAD: $P=5.56\times10^{-6}$; excluding CAD: $P=3.79\times10^{-5}$). Next, we sought to replicate the association between rs10757269 and ABI in both population-based and clinically-based samples (n=16 717). The magnitude and direction of the association in the replication studies was similar to the discovery set ($\beta=-0.0035$, $P=0.0176$), providing evidence of replication. In the combined stage 2 discovery plus replication meta-analysis, the ABI-rs10757269 association became stronger ($P=2.65\times10^{-9}$). The study-specific estimates of effect for the discovery studies, population isolates, replication studies, and overall discovery plus replication meta-analyses are presented in Figure 2. Two studies among the population isolates (the Amish Study and Croatia-Split) had effect estimates in the

Table 1. Meta-Analysis Results: ABI-SNP Associations with $P<10^{-5}$ in the Primary Discovery Analysis With Population Isolates Excluded

SNP	Chr	Physical Position	Closest Gene	Risk/Non-Risk Allele	Risk Allele Frequency	Meta-Analysis	N	Beta	SE	*P* Value	P_{het}
rs10757269	9	22062264	*CDKN2B*	G/A	0.49	ABI discovery	35 036	−0.0056	0.001	2.46E-08	0.23
						ABI replication	16 672	−0.0035	0.0015	1.76E-02	0.67
						ABI combined	51 708	−0.0049	0.0008	2.65E-09	0.38
						PAD† discovery	34 555	0.0849	0.0296	4.15E-03	0.32
rs4659996	1	238912747	*GREM2*	A/G	0.48	ABI discovery	28 087	−0.006	0.0012	4.44E-07	0.34
						ABI replication	16 658	−0.0018	0.0016	2.67E-01	0.65
						ABI combined	44 745	−0.0045	0.001	2.12E-06	0.32
						PAD discovery	27 574	0.0725	0.0319	2.31E-02	0.52
rs7003385	8	41705907	*ANK1*‡	T/C	0.67	ABI discovery	35 375	−0.0053	0.0011	5.24E-07	0.49
						ABI replication	16 690	−0.002	0.0016	2.20E-01	0.52
						ABI combined	52 065	−0.0043	0.0009	1.11E-06	0.43
						PAD discovery	34 903	0.0838	0.0314	7.57E-03	0.24
rs819750	1	99469651	*LPPR4*‡	G/T	0.12	ABI discovery	35 278	−0.007	0.0015	3.64E-06	0.51
						ABI replication	16 660	0.0022	0.0023	3.22E-01	0.99
						ABI combined	51 938	−0.0041	0.0013	1.01E-03	0.31
						PAD discovery	34 780	0.1068	0.0437	1.45E-02	0.06
rs9485528	6	102221473	*GRIK2*‡	A/G	0.17	ABI discovery	35 339	−0.0061	0.0013	4.63E-06	0.78
						ABI replication	16 679	0.0002	0.002	9.24E-01	0.63
						ABI combined	52 018	−0.0041	0.0011	1.77E-04	0.48
						PAD discovery	34 850	0.1172	0.0380	2.02E-03	0.80
rs722453	7	84037497	*SEMA3A*	G/A	0.42	ABI discovery	26 200	−0.0054	0.0012	6.43E-06	0.69
						ABI replication	6300	−0.0046	0.0025	5.74E-02	0.08
						ABI combined	32 500	−0.0052	0.0011	1.02E-06	0.59
						PAD discovery	25 706	0.0575	0.0318	7.05E-02	0.63
rs16824978	2	211380306	*CPS1*	T/C	0.25	ABI discovery	34 950	−0.0054	0.0012	7.77E-06	0.37
						ABI replication	14 340	0.0000	0.0019	9.94E-01	0.22
						ABI combined	49 290	−0.0039	0.001	1.48E-04	0.11
						PAD discovery	34 518	0.0760	0.0343	2.65E-02	0.39

P_{het} indicates *P* value for heterogeneity; ‡, SNP is located within the gene; rs819750 is within 60kb of the gene; †, PAD discovery: ABI <0.9 vs ABI >0.9. Chr indicates chromosome.

opposite direction to the other studies. None of the other SNP associations for ABI achieved genome-wide significance. The significance of the associations for the additional SNPs chosen for replication diminished in the discovery plus replication meta-analysis (Table 1, online-only Data Supplement Table VII).

PAD-SNP Associations

None of the SNP associations for the PAD phenotype (defined by an ABI ≤0.9) achieved genome-wide significance (Table 2; for meta-analysis results including population isolates see online-only Data Supplement Table VIII). The strongest association was found for rs6584389 on chromosome 10 near the *PAX2* gene (odds ratio 1.17, 95% confidence interval 1.10, 1.25, $P=2.34\times10^{-6}$). Of note, the chromosome 9 SNP rs10757269 association with PAD was in a direction consistent with the ABI association but did not achieve statistical significance (Table 1, $\beta=0.0849$, $P=0.004$, increasing the odds of PAD).

Overlap in SNP Associations for ABI and PAD

While the directions of effect for the ABI SNPs in Table 1 were consistent with the PAD association result (lower ABI, increased odds of PAD), there was little overlap in the top associations for the 2 phenotypes. Only 3 regions marked by SNPs in or near *IDE* (10q23–q25), *DAB21P* (9q33.2), and *GRAMD1C* (3q13.31), in addition to the chromosome 9p21 region, showed association with both ABI and PAD at the $P<10^{-4}$ level (online-only Data Supplement Table IX). SNP rs7100623 in *IDE* demonstrated the strongest novel association with both ABI ($\beta=-0.005$, $P=1.89\times10^{-5}$) and PAD ($\beta=0.139$, $P=8.39\times10^{-5}$) at $P<10^{-4}$; however, the association probability value was not significant in the replication stage, and diminished in the combined discovery plus replication meta-analysis.

Examination of PAD Candidate Genes

Among the 55 candidate genes or regions previously tested for association with ABI or PAD, 8 regions showed nominally significant probability values ($P<0.05$) after correction

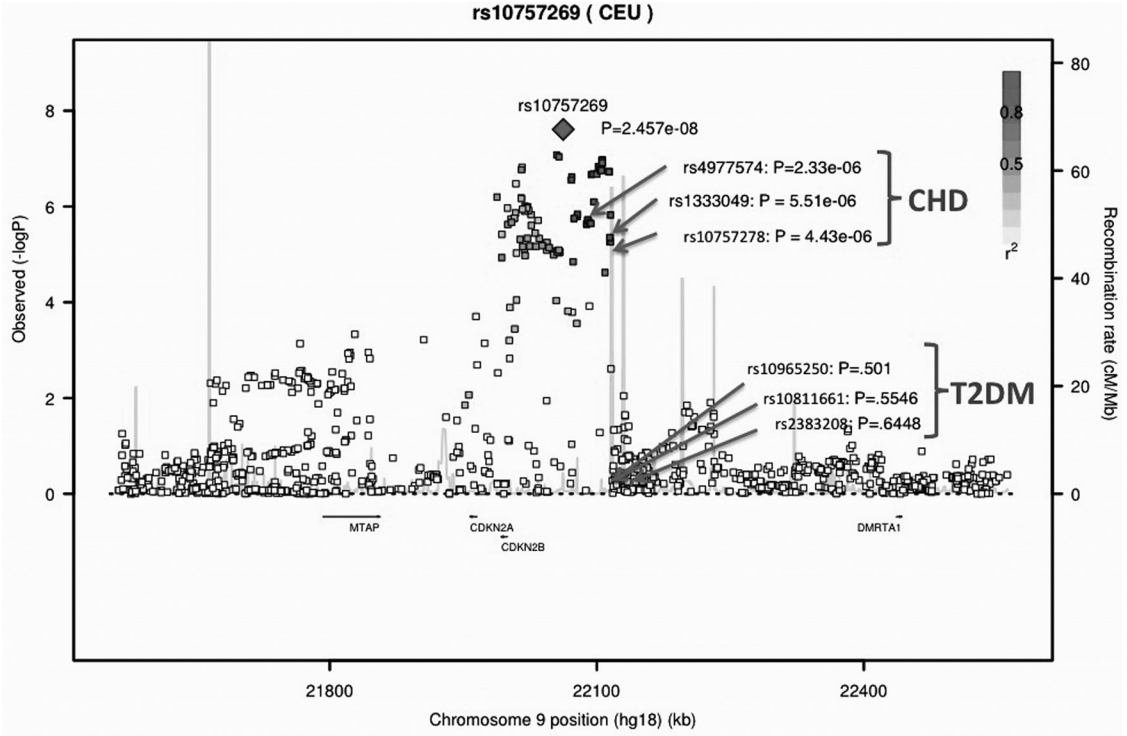

Figure 1. Genomic context of the genome-wide significant signal at chromosome 9p21 plotted against the $-\log_{10} P$ values. r^2 is between the top signal (rs10757269) and each SNP is shown. SNPs previously reported from genome-wide association studies (GWAS) to be associated with coronary artery disease (CHD, arrows), type 2 diabetes (T2DM, arrows), and *P* value for association with ankle-brachial index are shown. Chromosome positions are based on build hg18.

for the number of effective loci for each gene region. After accounting for the number of regions examined using a false discovery rate (FDR <0.10), we found evidence of association between ABI and *CYBA* (rs3794624, uncorrected $P=6.3\times10^{-5}$, corrected $P=0.0036$, FDR=0.0665) and *DAB21P* (rs13290547, uncorrected $P=3.6\times10^{-5}$, corrected $P=0.0035$, FDR=0.0665), in addition to the chromosome 9p21 locus (rs1333049) reported to be associated with ABI (Table 3).[35] We found no evidence of association between ABI and any of the other candidate genes previously tested for association with ABI or PAD (online-only Data Supplement Table X).

Examination of Coronary Artery Disease/ Myocardial Infarction Candidate Genes

Among the 30 SNPs previously reported by GWAS to be associated with CAD or myocardial infarction, 28 SNPs were available in our discovery meta-analysis of ABI, and 2 of these SNPs demonstrated an association (FDR <0.10) with ABI, including rs4977574 near *CDKN2B* ($P=2.33\times10^{-6}$) and rs1122608 in *LDLR* ($P=0.0026$) (Table 3, online-only Data Supplement Table XI).

Discussion

Our GWAS meta-analysis for ABI conducted in more than 40 000 adults of European ancestry has several notable findings. First, we identified and replicated 1 genome-wide significant association between a SNP in the chromosome 9p21 region and ABI. No other ABI-SNP associations achieved genome-wide significance. Second, in our discovery sample, over 3000 adults had PAD (ABI ≤0.9); however, none of the SNP associations were significant. Third, the directions of effect were consistent across the 2 phenotypes for the most significant ABI SNPs (lower ABI, increased odds of PAD): however, we observed minimal overlap in the top SNP associations for ABI and PAD. Finally, the effect size for the 9p21 SNP was modest. The association itself is, however, intriguing, and may provide insights into the biological mechanisms contributing to generalized atherosclerosis.

Chromosome 9p21 Locus and Atherosclerosis Susceptibility

Common genetic variants in the 9p21 locus are associated strongly with myocardial infarction and CAD,[17,33,36] and confer risk for other atherosclerotic diseases including stroke,[19] cerebral and abdominal aortic aneurysm,[20,21] and clinically diagnosed PAD; however, the relation with PAD was diminished when coronary artery disease cases were excluded.[20] SNP associations at the 9p21 locus with subclinical measures of atherosclerosis have been conflicting. Initially, no association was observed with carotid intima-medial thickness or flow mediated dilation in young or older adults;[37,38] however, more recent reports demonstrate an association with the development and progression of carotid atherosclerosis[39] and with the suggestion of a stronger effect in men.[40] To further investigate the ABI-9p21 SNP association noted in this study, we conducted the meta-analysis after adjusting for CAD and after exclusion of individuals with CAD. Not surprisingly, the association persisted but was no

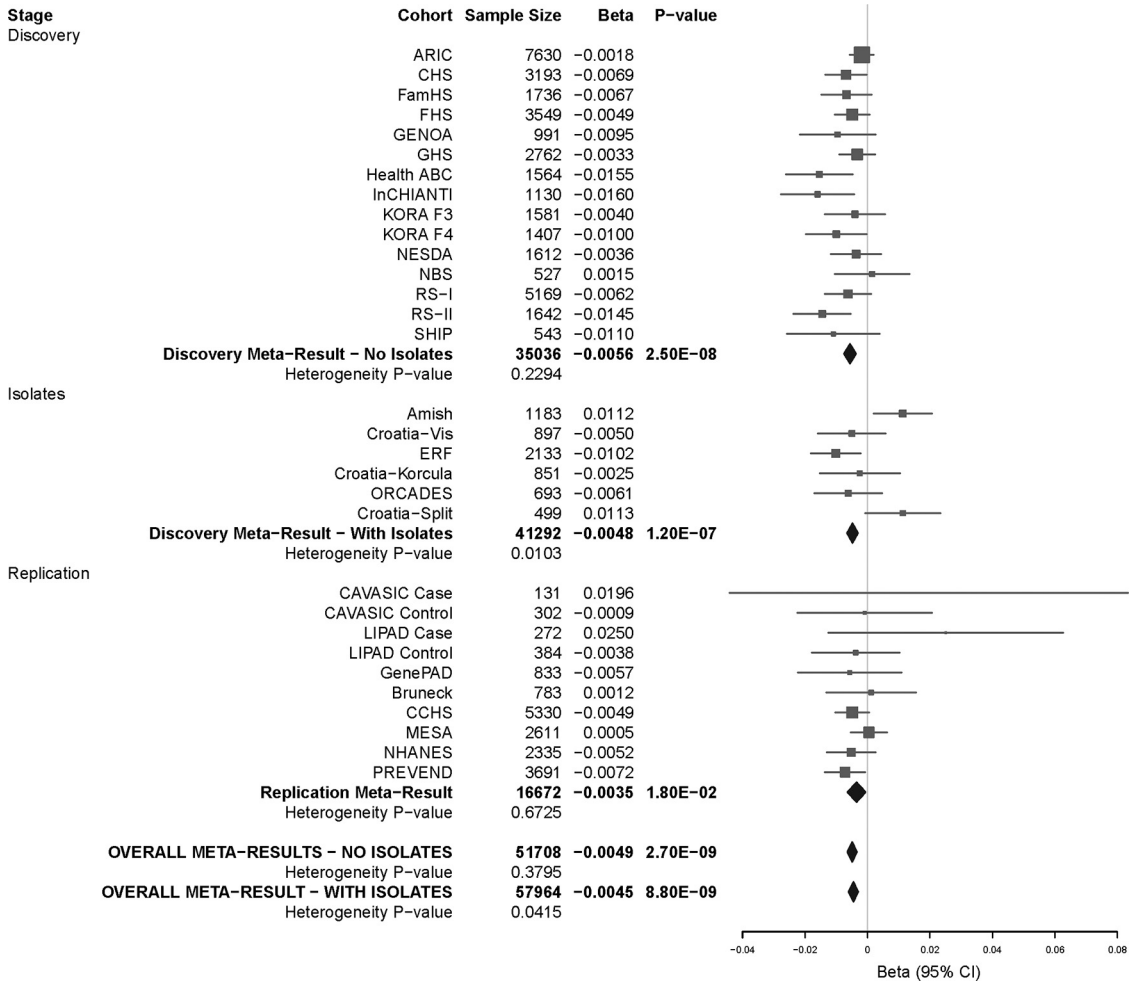

Figure 2. Ankle-brachial index-chromosome 9p21 (rs10757269) association: study-specific estimates of effect for the discovery studies, population isolates, replication studies, and overall discovery and replication meta-analyses.

longer genome-wide significant. Both CAD and PAD are manifestations of underlying atherosclerosis, and nearly two thirds of individuals with PAD have coexisting coronary or cerebrovascular disease.[41] One previous report conducted in 3 studies of older adults identified an association between a variant at 9p21 and lower ABI, as well as an increased risk for PAD.[35] The primary effect of the chromosome 9p21 region may be on the atherosclerotic process itself, and there are likely to be many other factors, both genetic and environmental, that determine whether it manifests as CAD, PAD, or another clinical atherosclerotic phenotype. The primary biological mechanism underlying the association with ABI is unknown but appears to be independent of 2 major PAD risk factors, diabetes and smoking, as the ABI SNP in the 9p21 region we identified is not in linkage disequilibrium with the SNPs in the region associated with diabetes risk[42,43] or smoking-related behaviors.[44] The mechanism may be related to modulation of platelet reactivity,[45] atheroma formation, plaque instability, thrombosis, or biological processes not yet identified.[46] The SNP associated with ABI is nearest to

Table 2. Meta-Analysis Results: SNP Associations for PAD (ABI ≤0.9 vs ABI >0.9) With $P<10^{-5}$ With Population Isolates Excluded

SNP	Chr	Physical Position	Closest Gene	Risk/Non-Risk Allele	Risk Allele Frequency	N	OR	95% Confidence Interval	*P* Value	P_{het}
rs6584389	10	102459392	*PAX2*	C/A	0.50	24 474	1.17	(1.10, 1.25)	2.34E-06	0.37
rs9998941	4	162544312	*FSTL5**	A/G	0.23	34 670	1.18	(1.10, 1.27)	2.34E-06	0.61
rs11751656	6	42751046	*UBR2**	G/A	0.07	27 470	1.61	(1.32, 1.96)	2.46E-06	0.75
rs4535726	8	68938371	*DEPDC2*	T/C	0.20	34 915	1.18	(1.10, 1.26)	3.79E-06	0.01
rs2090205	17	73897869	*PGS1**	A/C	0.24	34 912	1.16	(1.09, 1.24)	5.01E-06	0.17
rs11933540	4	25729099	*RBPJ*	C/T	0.30	34 830	1.15	(1.08, 1.23)	9.86E-06	0.08

P_{het} indicates *P* value for heterogeneity.
*SNP is located within the gene. Chr indicates chromosome.

Table 3. Literature-Reported Candidate Genes for Peripheral Artery Disease and Coronary Artery Disease and Their Association With Ankle-Brachial Index in the CHARGE GWAS Discovery Sample (Population Isolates Excluded) With FDR <0.10†

SNP	Chr	Physical Position	Closest Gene	Risk/ Non-Risk Allele	Risk Allele Frequency	N	Beta	SE	*P* Value*	# of effective loci‡	*P* Value Corrected‡	False Discovery Rate‡
PAD genes												
rs10757269	9	22 062 264	*CDKN2B*	G/A	0.51	35036	−0.006	0.001	2.50E-08	69	1.70E-06	9.32E-05
rs3794624	16	87 244 575	*CYBA*	G/A	0.34	31035	−0.005	0.001	6.30E-05	58	3.60E-03	0.0665
rs13290547	9	123 527 316	*DAB2IP*	T/C	0.06	32135	−0.009	0.002	3.60E-05	97	3.50E-03	0.0665
CAD genes												
rs4977574	9	22 088 574	*CDKN2B*	G/A	0.49	35411	−0.0047	0.001	2.33E-06	. . .	. . .	6.52E-05
rs1122608	19	11 024 601	*LDLR*	G/T	0.74	35384	−0.0035	0.001	2.56E-03	. . .	. . .	0.036

**P* value from Discovery GWAS of ABI. Chr indicates chromosome.

†Candidate genes for PAD were selected for testing with ABI if an association study with at least 100 cases and 100 controls was available in the literature, independent of whether the study was positive or negative. Genes for CAD were considered only for testing with ABI if they were identified by recent GWAS to be genome-wide significantly associated with CAD. The table shows only the genes which showed an experiment-wise significant association with ABI after correction for multiple testing. The entire list of genes can be seen in online-only Data Supplement Table X and XI for PAD and CAD genes, respectively.

‡Due to the high correlation of imputed genotype scores, the effective number of loci was calculated for each PAD gene region (31) using the genotype scores from the KORA F4 Study. Bonferroni correction of *P* values then was applied in each region using this number. Furthermore, the corrected *P* value thresholds of significance for 28 CAD loci (tested in online-only Data Supplement Table XI, $\alpha=0.05/28$, 1.85×10^{-3}) and 55 PAD loci (tested in online-only Data Supplement Table X, $\alpha=0.05$/effective number of loci) were calculated. We also calculated a false discovery rate (FDR) using the corrected *P* values accounting for the number of gene regions examined. An FDR <0.10 defined evidence of a significant association.

CDKN2B, a well recognized tumor-suppressor gene that encodes a cyclin-dependent kinase inhibitor and is involved in regulation of the cell cycle. *CDKN2B* is abundantly expressed in human atherosclerotic lesions,[47] and animal models suggest that altered *CDKN2A/B* expression results in abnormal regulation of vascular cell proliferation.[48] Functional studies reveal a long noncoding RNA at this locus named ANRIL, and a mouse model has confirmed the essential role of ANRIL in regulation of *CDKN2B* expression through a cis-acting mechanism.[49,50] ANRIL is implicated in proliferation and senescence.

PAD Candidate Genes

We performed a literature search to identify all candidate gene regions previously investigated for association with PAD or ABI, irrespective of whether the association was reported to be positive or negative. This approach revealed 2 further associated gene regions: *DAB2IP* and *CYBA*. *DAB2IP* rs13290547 was not only associated with ABI, but also with PAD ($P=3.62\times10^{-5}$ and 2.2×10^{-5}, respectively; online-only Data Supplement Table X). The *DAB2IP* gene encodes an inhibitor that is involved in the regulation of cell survival and proliferation. One variant in the *DAB2IP* gene (rs70254486) recently has been detected in a GWAS of abdominal aortic aneurysm.[51] That study also detected an association with PAD as a secondary end point in 3690 cases versus 12 271 controls ($P=3.9\text{x}10^{-5}$). The same SNP showed an association with CVD within a meta-analysis of case control studies.[52] The *CYBA* gene is involved in NADPH oxidase regulation, which contributes to oxidative stress and plays a key role in the pathophysiology of coronary disease. Only 1 report investigated a SNP (rs4673) in this gene for association with PAD among 324 cases and 295 controls, but did not find an association.[53] Our study found an association of rs3794624 ($r^2=0.5$ with rs4673) with continuous ABI, which may indicate that the earlier study likely lacked power to find this association. None of the other gene regions had sufficient evidence for association with continuous ABI in our meta-analysis. Another very wide-reaching approach designed to systematically examine a large number of genes related to intermediate phenotypes of atherosclerosis, such as blood pressure regulation, lipoprotein metabolism, inflammation, oxidative stress, vascular wall biology, obesity, and diabetes, found only eNOS to be significantly associated with ABI.[14] This gene could not be confirmed by our candidate gene examination.

Coronary Candidate Genes

Besides the chromosome 9 locus, 1 other SNP reported to be associated with coronary disease in recent GWAS also showed an association with ABI in our study; rs1122608 in *LDLR*. The *LDLR* gene plays an important role in cholesterol homeostasis, and mutations at this gene have been shown to influence LDL cholesterol levels and the subsequent risk for coronary disease.[54] The association of *LDLR* gene with ABI in our study is a confirmation of the shared biological pathways underlying both subclinical and clinically apparent disease.

Strengths/Limitations

Our meta-analysis represents the largest collaborative effort to date to identify genome-wide SNP associations for variation in ABI and PAD (ABI ≤0.90), and our findings suggest the absence of common variants with large effects on ABI. Use of ABI as our primary phenotype has major advantages of detecting asymptomatic PAD, as the ABI is an objective measurement, whereas clinical PAD requires subjective symptoms of exertional leg discomfort and mobility of the individual. However, several limitations of our meta-analysis merit comment. The blood pressure measurement protocol and ABI calculation was heterogeneous across participating studies. While protocols were standardized within each study,

the studies were not designed to be fully standardized and comparable across studies (online-only Data Supplement Table I). This phenotype heterogeneity may have impacted our ability to detect associations. Furthermore, for many studies, information about a previous revascularization intervention was not available. This lack of data may have resulted in the misclassification of some of the most affected persons by placing them into an ABI range of unaffected individuals and consequently reducing our power to detect true associations. Our sample was restricted to individuals of European ancestry, and thus our findings cannot yet be generalized to individuals of other race or ethnic groups. Furthermore, some PAD susceptibility variants may be race or ethnic specific and only can be uncovered through the study of non-Europeans. For example, African-Americans have a higher prevalence of PAD that cannot be attributed to traditional or novel risk factors.[55] This observation raises the hypothesis that polymorphisms unique to African-Americans partially may be responsible for the higher prevalence of PAD.[55] We did not evaluate gene by environment interactions, which may be especially relevant for cigarette smoking, a strong risk factor for PAD,[56] and a factor known to interact with other genes to modulate atherosclerosis.[57]

Conclusions

In conclusion, a common variant near the *CDKN2B* gene in the chromosome 9p21 locus is associated with a lower ABI. PAD represents a diffuse form of atherosclerosis associated with increased risk for death and incident CVD events. Thus, the identification of genetic variants associated with ABI may provide an important opportunity not only to unravel the biological basis of PAD, but also to improve our understanding of the causes of the variation in degree of atherosclerosis from 1 arterial bed to another. Additional studies are warranted to identify the causal variants in the 9p21 locus and to characterize their functional significance. The search for genes influencing predilection to PAD remains elusive, and alternative approaches are warranted.

Appendix

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None.

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CLINICAL PERSPECTIVE

Little is known about the genetic susceptibility to peripheral arterial disease (PAD). We conducted a meta-analysis of genome-wide association study findings for the ankle-brachial index (ABI), a noninvasive measure of PAD, within an international consortium of 21 population-based cohort studies that included over 40 000 participants of European descent, and conducted replication analyses in over 16 000 individuals from population-based cohorts and clinically-based studies of PAD. We identified and replicated 1 genome-wide significant association between a genetic variant in the chromosome 9p21 region and a lower ABI. Common genetic variants in the 9p21 locus are associated strongly with coronary artery disease and confer risk for other atherosclerotic diseases. Therefore, the primary effect of the 9p21 region may be on the atherosclerotic process itself, and there are likely many other factors, both genetic and environmental, that determine whether it manifests as coronary disease, PAD, or another clinical atherosclerotic phenotype. The primary biological mechanism underlying the association with ABI is unknown but appears independent of 2 major PAD risk factors, diabetes and smoking, as the ABI single nucleotide polymorphisms (SNP) in the 9p21 region we identified is not in linkage disequilibrium with the SNPs in the region associated with diabetes or smoking-related behaviors. PAD represents a diffuse form of atherosclerosis associated with increased risk for death and incident CVD events. Identification of genetic variants associated with ABI may provide an opportunity to unravel the biological basis of PAD.